Intramuscular Vascular Endothelial Growth Factor Gene Therapy: Fact or Fiction?

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Somatic gene therapy for the cardiovascular system has been an active area of investigation since Nabel's report on vascular cell transfection using viral vectors (1). In theory, replacement of missing gene products (e.g., deficiency of hepatic low-density lipoprotein receptors in familial hypercholesterolemia), increasing production of molecules to counteract a disease process (e.g., growth factors to stimulate angiogenesis), and limiting gene expression (e.g., cell cycle proteins to attenuate smooth muscle cell proliferation) are potential applications. The most promising use has been observed in animal models of intramuscular gene transfer for hind limb or myocardial ischemia, in which angiogenic growth factors, such as fibroblast growth factor (FGF)-1, FGF-4, vascular endothelial growth factor $(VEGF)_{121}$, $VEGF_{165}$, VEGF-C, and hypoxia-inducible factor-1, were employed to promote perfusion and collateral artery development (2–4). Wolff and colleagues have demonstrated that intramuscular injection of naked plasmid deoxyribonucleic acid (DNA) is also feasible (5), and they have suggested that striated muscle might be suitable for heterologous transgene expression. This strategy obviates immunological concerns about viral vectors, and because plasmid DNA remains in a nonreplicative, unintegrated form, this approach is unlikely to be complicated by insertional mutagenesis.

In this issue of *The American Journal of Medicine,* Shyu et al. (6) describe the use of intramuscular $phVEGF_{165}$ gene transfer in patients with severely ischemic limbs. Their results support those of Isner et al. (7). An increase in plasma VEGF level indicates active transgene expression, and development of peripheral edema reflects bioactivity. That VEGF was not associated with substantial edema supports the suggestion that the permeability-enhancing effects of VEGF need to be potentiated by tissue ischemia (8) The lack of a relation between plasma VEGF level and a therapeutic effect probably reflects inconstant washout of VEGF from the ischemic limb.

The most important finding was that of a dose-related effect, in which a minimum of 2400 μg of $phVEGF_{165}$ was needed to induce robust, clinically relevant neovascularization, with further clinical and hemodynamic improvement observed after administration of 2000-μg $phVEGF_{165}$ booster injections. Isner et al. (7) had reported new blood vessel development with an initial dose of 100 μg of $phVEGF_{165}$ and similar dose escalation to 2000 μg. They also noted spider angioma on the ankle and foot, which developed about a week after gene therapy but regressed spontaneously. Light microscopy of a biopsy specimen showed a markedly increased diameter of the vascular lumen, similar to supernumerary vessels ascribed to overexpression of VEGF in an avian embryo model (9). That VEGF has different effects depending on concentration and microenvironment was suggested later (10).

Angioma-like vessel proliferation in skeletal muscle has been reported in a murine model of myoblast-mediated overexpression of VEGF (10). Continuous, constitutive overexpression of VEGF was due to ex vivo retroviral gene transfer to myoblasts that were transplanted into nonischemic skeletal muscle. Unregulated VEGF expression led to a highly localized formation of disorganized vessels that closely resembled those in tumors or early vasculogenesis. Because angioma-like structures rarely increased collateral development after a transient exposure in animal models of myocardial or hind limb ischemia, it was suggested that angiogenesis may prevail with low concentrations under tissue hypoxia, whereas a deleterious response resulting in an immature vascular network may occur with high concentrations under nonischemic conditions. More recently, Dor et al (11) provided new insights into conditional switching of VEGF expression to circumvent embryonic lethality in a transgenic mouse model. Increased expression led to unlimited branching of the existing vasculature, increased vessel density, and formation of vessels that had abnormally large lumens and were irregularly shaped. Stimulated neovascularization was not self-limiting or limited by the exhaustion of downstream factors. Most importantly, premature cessation led to the regression of new vessels, whereas a delayed switch-off made vessels refractory to VEGF withdrawal and regression; thus, questioning the utility of therapeutic approaches that rely on short duration of stimulus. Overexpression also promoted the formation of large vessels, some of which were coated with smooth muscle cells. Hence, VEGF was shown to also support the recruitment of periendothelial cells, confirming the need for a single-factor-based proangiogenic therapy.

Owing to the complex dose-effect relation, it is important to document accurately the development of func-

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tionally sufficient neovascularization. Angiography lacks the quantitative precision and reproducibility to study small collaterals. Furthermore, it may not be sensitive enough to detect perfusion of the nutritive microcirculation. Measurement of peripheral blood pressure may be inaccurate, and the ankle-brachial index does not always parallel nutritive perfusion changes. An approach to assess the relative differences in skeletal muscle perfusion in patients with or without arterial occlusive disease involves measurement of the deoxygenation and reoxygenation of myoglobin by proton magnetic resonance spectroscopy of deoxymyoglobin (12). This technique, adapted from strain gauge plethysmography to measure reactive hyperemia, circumvents the problem that the resting blood flow rate of muscle is so low that it does not accurately reflect the degree of ischemia and perfusion changes. This method has also been shown to be more accurate than strain gauge plethysmography (12).

The evaluation of angiogenic gene therapy in patients is further complicated by the observation of spontaneous neovascularization and collateral formation in animal studies. It is not known how these animal models relate to the clinical scenario in which there is decreased blood flow to the limbs for weeks to months. Indeed, it remains to be determined whether patients with chronic critical limb ischemia who are unsuitable for surgical or catheter-based revascularization are suitable for treatment with growth factor–induced neovascularization. These patients may have irreversible muscle and nerve damage. They are also more likely to have secondary local infections, as well as an increased risk of amputation, independent of perfusion changes.

The choice of endpoints in these trials is important, because at present there is no "gold standard" to document angiogenesis in humans. The Transatlantic Conference on Clinical Trial Guidelines in Peripheral Arterial Disease recommended that complete relief from pain without the use of analgesics, and complete healing of all ulcers of both legs and amputation rates, be the endpoints in randomized, double-blind, parallel-group trials (13). It is hoped that ongoing clinical trials involving patients with severe peripheral arterial occlusive disease will provide further information to confirm the potential that gene therapy has demonstrated thus far, and gaps in knowledge will not jeopardize attainment of outcomes in angiogenic gene therapy trials.

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